INFECTION OF COCKROACHES WITH HERPOMYCES (LABOULBENIALES). I. LIFE HISTORY STUDIES [1, 2]

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Largely by chance it was discovered that our laboratory colony of the oriental cockroach (*Blatta orientalis* L.) was infected with *Herpomyces stylopygae* Spegazzini. Subsequently, some of our boxes of the german cockroach (*Blattella germanica* L.) were found infected with *H. ectobiae* Thaxter. How long these infections have been present in our colonies is not known. More recently we introduced a colony of a giant tropical cockroach (*Blaberus craniifer* Burm.) from Key West, Florida; on arrival these specimens were found infected with *H. tricuspidatus* Thaxter. Since most past studies of the Laboulbeniales, and all of those on cockroach-inhabiting species, have been by taxonomic mycologists, we decided to examine the etiology and the host-parasite relationship from an entomological point of view. Incidentally, some points of strictly mycological interest were noted and will be recorded.

Although we have utilized all three of the above species of *Herpomyces,* the majority of our data deal with *H. stylopygae* on oriental cockroaches. The data are sufficiently voluminous that they are being presented as a series of papers. In the present paper we are presenting data on the life history of the fungus, its transmission, and some notes on the structure of asci and ascospores. In another paper (Richards and Smith, 1955a) we are presenting our histological and histopathological work. A separate note (Richards, 1954) treats histochemical differentiation of the fungal walls. Subsequent papers are planned covering our experimental work on host specificity, location on the host specificity, germination and development.

Due to the tremendous variability within the Laboulbeniales, one should remember that all of our data deal with the single genus *Herpomyces* found only on cockroaches, and most of our data deal with the single species *H. stylopygae.* The majority of the approximately 1500 described species (Benjamin and Shanor, 1950) of Laboulbeniales occur on species of Diptera and Coleoptera (flies and beetles). Morphology and habits are quite diverse. For instance, there are a number of records of spores germinating while still within the perithecium

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(*Laboulbenia*, *Dioicomyces*, *Dimorphomyces*, etc.); with thousands of specimens examined we have never seen this phenomenon in *Herpomyces*. Generalization seems dangerous at the present stage of knowledge of this group.

The Validity of Herpomyces stylopygae Spegazzini

Thaxter (1908) described the species *H. periplanetae* as occurring on both the american (*Periplaneta americana* L.) and the oriental (*Blatta*, = *Stylopyga*, *orientalis* L.) cockroach. Subsequently, Spegazzini (1917) separated the form found on the oriental cockroach on the basis of relatively slight differences: larger perithecia, a blunter shield over the secondary receptacle, a blackened base to this shield (Fig. 3) and a few other minor points. Thaxter (1931) questioned this separation on such slight differences, but we have had to conclude that the two are distinct (whether species or strains) because we have been unable to obtain development of the form from oriental cockroaches on *Periplaneta americana*, *P. australasiae* or *P. brunnea*, the listed hosts of *H. periplanetae* (details will be given in Richards and Smith, 1955b). Yet there is no difficulty in transferring the infection in the laboratory from one oriental cockroach to another.

Distribution of H. stylopygae on Blatta orientalis

The infection is most commonly limited to the antennae (Fig. 1). Of 50 infected cockroaches taken at random from the colony and examined carefully, 38 (75%) had plants only on the antennae, 11 (22%) had heavy antennal infections plus some plants on the maxillary palpi, and a single specimen (2%) had a heavy antennal infection plus some plants on both the maxillary and labial palpi. No plants were found elsewhere on the body. However, with hundreds of roaches handled (but not searched) subsequently we have found several males with a few plants on the cerci, and one male with four plants growing close to one another on the ventral surface of the right metathoracic femur. In no case have plants been found elsewhere on the body except when there was a very heavy infection on the antennae. And nowhere on the body except on the antennae do the infections become so heavy they almost hide the surface of the infected part. The antenna shown in Figure 1 represents only a moderate infection; heavy infections so cover the antennae that they photograph less well.

The long antennae of cockroaches are their investigative organs. In a colony, and in nature, they are waved about in all directions making repeated contact with their substrate and their neighbors. When under crowded conditions, common in nature as well as in culture boxes, cockroaches are continually making contact with the antennae of other individuals. The contact involves all exposed surfaces, even the ventral surface, as one cockroach runs over another. As shown in Figures 1 and 2, mature perithecia commonly have a spore or spore group protruding from the subterminal aperture for 80–85% of its length. Such spores are dislodged by a touch or jarring. Considering the habits of cockroaches and the presence of such easily dislodged spores, it is obvious that the antennae must serve as efficient spore brushes and spore dusters. Further, it seems obvious that under crowded conditions spores should become placed on all portions of the body. It is simple to demonstrate that this is indeed true; areas of the cockroach body wall

FIGURE 1. A small portion of an antenna of *Blatta orientalis* moderately infected with *Herpomyces stylopygae* (living). There are fifteen plants on these six antennal segments. Note spores protruding from the subterminal apertures of many of the perithecia.

FIGURE 2. Higher magnification of mature, living perithecia showing a spore or pair of spores (arrow) protruding from each perithecium (only one in clear focus).

FIGURE 3. Basal shield of *H. stylopygae* showing naturally blackened base and stained apex (cotton-blue). Note also pair of spores on cuticle surface (one in 2-cell stage, other in 4-cell stage. Bar 10 μ.

FIGURE 4. Young mature female plant of *H. ectobiae* on antennal seta of *Blattella germanica*. Note basal holdfast of spore (arrow) and basal prolongation of plant to setal base. Cotton-blue stain. Bar 10 μ.

FIGURE 5. Larger female plant of *H. ectobiae* spreading across antennal surface of *B. germanica* after reaching base of seta (S). Cotton-blue stain. Bar 10 μ.

FIGURE 6. Young male plant of *H. ectobiae* on antennal seta of *B. germanica*. Note spore in 4-cell stage alongside this plant on seta (holdfasts indicated by arrows). Cotton-blue stain. Bar 10 μ.

excised, stained with cotton-blue in lacto-phenol and examined under the microscope show spores all over the body surface. Clearly the hypothesis that the specific locations of infections are due to transfer of spores to only those locations, as suggested by Thaxter (1896) and preferred by Benjamin and Shanor (1952) for *Laboulbenia* species, cannot hold for infections of *H. stylopygae*. We hope at a later date to discuss this question of development to maturity only on certain areas, but we might mention here, in passing, that in single insect species histochemical differences in the cuticle surface for different areas of the body have recently been reported (Richards, 1952).

At frequent intervals, cockroaches clean their antennae by bending them down and pulling the shaft of the antenna through the mouth parts, thus masticating and scraping them. Likely this is the source of infections developing on palpi though infection from another individual does not seem impossible. It is tempting to speculate that the lower incidence of infection on palpi may be due to the antennal infection having to mature first but the very low incidence on all parts of the body except the antennae makes such a speculation decidedly questionable.

On the antennae plants may be located anywhere but are seldom seen on the most basal segments. On the average there are about one hundred segments in each antenna of *B. orientalis*. Approximately equal numbers of plants grow to maturity on segments of the apical 90% of the antennae, more local distribution in individual cases being shown by actual counts of spores and plants to be correlated with and hence due to corresponding irregularities in the chance distribution of spores.

Plants may be found growing on hard sclerite areas or on soft membrane. Correlated with the fact that most of the surface of the antenna is sclerotized, most of the plants are found on sclerites. Commonly they are associated with setae and penetrate through the setal sockets but also, commonly, they penetrate through sclerite where no seta is located (see Richards and Smith, 1955a).

Infections develop much more heavily on males than on females. Rough estimates from 24 ♂ and 24 ♀ taken from infected colonies show that there are on the average more than twice as many plants on infected males. Infections on nymphs are never very heavy but infected individuals of all instars can be found. In a specific test where 4 ♂, 4 ♀ and 4 nymphs were exposed to infection from 8 adult males (in a pint jar) for just one day and then isolated, 117 mature plants were found on the males, 62 on the females but only 11 on the nymphs at about two weeks later. For these counts, both antennae were amputated at the base, stained with cotton-blue in lacto-phenol and the number of spores and plants tabulated for every antennal segment. It was found that there were 416 spores or plants on the females and 525 on the males, *i.e.*, about 50 per antenna, but only 119, *i.e.*, 15 per antenna, on the nymphs. Since infection was from adult males, it follows that infected males distribute approximately equal numbers of spores to adults of either sex but significantly less to nymphs. Of more interest is the fact that exposure to infection was on a known single day and examination was made at a known subsequent interval (11–14 days). If one assumes all spores will germinate and grow on an antenna, all of these should have been reaching maturity at the time of examination. Tabulation was made under four headings: 2-cell spores, 4-cell spores, immature plants, and mature plants. Of the total of 1060 spores, only 16 were still in the 2-cell stage, but 502 had not progressed past

the 4-cell stage. This growth failure of almost 50% was not uniformly distributed. Tabulating the values:

on nymphs (119 spores): 29% past 4-cell stage, 9% mature, at 13–14 days,
on females (416 spores): 44% past 4-cell stage, 15% mature, at 12–13 days,
on males (525 spores): 67% past 4-cell stage, 22% mature, at 11–12 days.

Clearly, either the per cent growing on males > on females > on nymphs, or there is a delay at the 4-cell stage with sporadic initiation of subsequent development being most frequent on males and least frequent on nymphs. At this 11–14 day time almost exactly one-third of those which had passed the 4-cell stage had mature perithecia in each group. Perhaps this can be interpreted as favoring the second alternative. Such a difference in frequency of initiation of later development would give the observed point that, both in colonies and after known infections, about twice as many *mature* plants are seen on males as on females.

We have succeeded in inducing growth of *H. stylopygae* only on live cockroaches. Plants will not grow past an early germination stage on exuviae (shed skins) or on excised and explanted pieces of abdominal cuticle or integument floating on broth in a humid atmosphere; they will not grow on amputated antennae even at 100% relative humidity; and if the cockroach dies the plants wither within a few days. However, plants are active for some time after infected antennae are amputated. An amputated antenna makes a good spore brush for experimental infections. After one use it may be held for a few hours in moist air and used again. Actually this is illustrated by Figure 1. In mounting this amputated antenna for photographing, all the spores were dislodged; the mount was held in moist air and this photograph taken some hours later.

Following infection with spores, mature *H. stylopygae* are found on *Blatta orientalis* in a little less than two weeks (25–27° C.). About the same length of time is required for *H. ectobiae* on *Blattella germanica*. Somewhat longer, perhaps three weeks, is required for *H. tricuspidatus* on *Blaberus*.

Egg capsules from heavily infected *B. orientalis* females give batches of nymphs which do not develop infection. Also spores dusted or brushed onto egg capsules or exposed eggs do not develop into visible plants. Finally, surgical implantation of infected antennal fragments into various parts of the body cavity of oriental cockroaches gave entirely negative results.

In conclusion, infection is readily obtained by direct contact placing the spores on an appropriate external surface but, in our experience, by no other means. It is reasonable to suppose, as Thaxter (1896) did, that infection might also be indirect by deposition of a spore on any surface from which another insect by chance collects it onto itself. Lindroth (1948) has recently reported such indirect transmission in certain *Laboulbenia* spp. where apparently spores may survive in soil for some weeks before being picked up by a passing beetle. We have not attempted to check this possibility but to judge from our experience with glass needles (Table I) we would suspect that while many spores are doubtless dropped, few would ever subsequently become transferred to a passing insect.

Notes on Asci and Spores of H. stylopygae

Having only dry material at his disposal, Thaxter was unable to be sure how many spores were in an ascus. Most Laboulbeniales have four spores per ascus

but some have eight. Clearly there are eight in *H. stylopygae* (Figs. 7 and 8). The asci are a fat cigar-shape and measure about $12 \times 60\ \mu$, those used for the photographs seeming to be broader because somewhat flattened by cover-glass pressure to bring the spores into one focal plane.

An apparently new point is that in this species the asci do not disintegrate to liberate spores within the perithecia. Perithecia of living plants teased in a drop of water liberate both spore-filled and empty asci (Figs. 8 and 9). The spore-filled asci were sometimes seen to liberate their spores one after another through

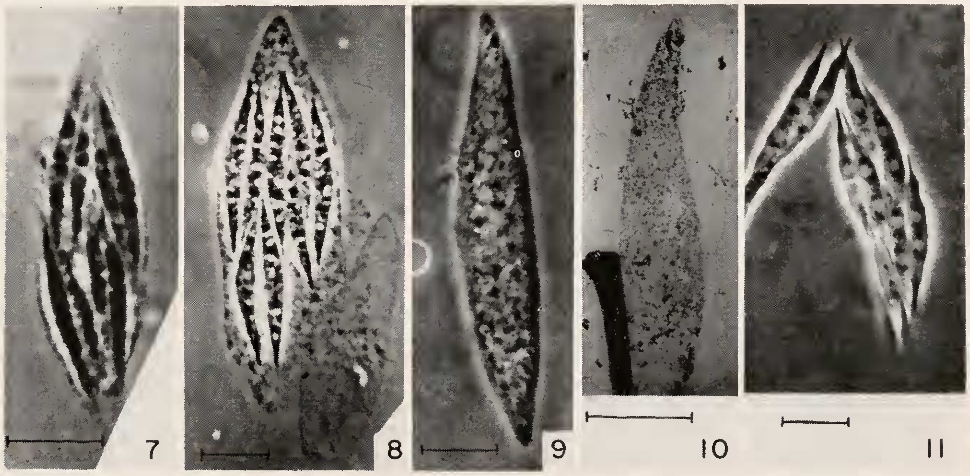

FIGURE 7. Mature ascus with its eight spores after staining with the gram stain. From perithecium of *H. stylopygae*. Note that both spores and the ascus wall are gram positive. Bar 10 μ.

FIGURE 8. Mature ascus of *H. stylopygae* in water; somewhat compressed by coverglass pressure. Dark phase-contrast photomicrograph of living material. The pressure used to get all the spores into one focal plane has resulted in a rupture of the ascus wall and extrusion of material at the lower right of the figure (such rupture is not normal). Bar 10 μ.

FIGURE 9. Empty ascus or ascus ghost of *H. stylopygae* in water. Dark phase-contrast photomicrograph (not stained). Note contents, absence of any indication of rupturing or disintegration (spores released through terminal pore), absence of any operculum, and apparently unitunicate condition. Bar 10 μ.

FIGURE 10. Electron micrograph of empty ascus ghost of *H. stylopygae*. Shows extreme thinness of dry ascus wall, and hence small amount of solid material therein, and no indication of more than a single layer. Bar 10 μ.

FIGURE 11. Living spores of *H. stylopygae* in water; teased from fresh perithecium. Note clustering of the spores. Bar 10 μ.

an aperture in the end as drying of the water began to exert cover-glass pressure on the ascus. No rupture of the ascus wall was evident, and the terminal hole is not visible after the spores have been discharged (Fig. 9). Although the observed liberation of spores was under unnatural condition, the presence of numerous empty asci within partially emptied perithecia implies that liberation of the spores is normally via a perforation (presumably at the distal end) rather than by disintegration. As Figures 9 and 10 show, the asci are inoperculate and unitunicate (see Luttrell, 1951).

The spores seem to be always liberated from the asci while within the perithecia. At least we have never seen asci ejected, the canal of the perithecium is too small for an intact ascus, unless the ascus were compressed, and we obtained only spores, never asci, in picking spore groups off the tip of the perithecium (Table I).

After liberation of the spores, the empty ascus is left as a ghost containing many highly refringent granules and droplets (Fig. 9). Commonly, active Brownian movement is evident. Dr. Benjamin suggests that these granules and droplets represent epiplasm, *i.e.*, protoplasm remaining after the aggregation of most of the material into spores (by "free cell formation"). The interior of mature perithecia contains gram-negative, bipolar-staining, short-rod, bacteria. In stained smears these are particularly abundant around empty or torn asci.

The ascus wall, like the spores, is gram-positive, and of the order of 0.1 μ thick. On electron microscope grids the empty asci dry down to give ghosts of about $9 \times 43\ \mu$; correcting for the flattening this gives about $6 \times 40\ \mu$. The electron micrographs show only that the ascus wall is exceedingly thin after drying, not over a few hundredths of a micron thick, and that it is heavily contaminated with debris, presumably from the granule-containing fluid within the empty ascus (Fig. 10).

It is generally stated that spores are discharged in pairs throughout the Laboulbeniales although various published drawings of asci do not show such association of spores in pairs. Dr. R. K. Benjamin tells us that there is good evidence to believe that this generality holds for many genera because the dioecious plants are invariably found growing in pairs even though widely scattered on the host (*Dioicomyces, Aporomyces, etc.*).[3] However, Thaxter (1931) has already remarked on the commonness of single plants in *Herpomyces,* and it became evident early in our studies that this was partly or perhaps entirely due to the discharge of single spores. Single plants could also originate from the loss of one member of a spore pair; we do not know whether this occurs naturally but in lacto-phenol mounts one sometimes sees two sets of holdfasts but only a single spore (Fig. 13), indicating the loss of one member of the pair either before or during preparation of the slide.

To determine the actual number of spores discharged at one time, a census was made of the spore clusters protruding from the perithecia of 128 ($= 2^7$) plants. For this purpose, infected oriental cockroaches were observed under high power of a dissecting binocular microscope, protruding spore groups picked off individual perithecia by touching the group with the tip of a fine glass microneedle, and then transferring the spore group to a droplet of cotton-blue stain in lacto-phenol. Subsequently the coverglasses bearing rows of stain droplets were inverted on a depression slide and each droplet carefully searched, with the final check on spore count being made under oil immersion. Validation of the technique was observational: the protruding spore groups could be seen to be removed;

[3] This opinion assumes that all spores germinate and grow into readily located plants. An alternative possibility is that plants mature only when in pairs. Some reason for holding the second possibility in mind until it is disproven comes from data on the germination of *Herpomyces* on hosts on which they will not mature. These data will be presented subsequently. Also we have occasionally seen single plants of *H. ectobiae* alongside an ungerminated spore; these could have arisen from growth of only one of a spore pair (Fig. 6).

they could be seen on the microneedle, and the microneedle was clean following immersion in the stain droplet.

The data may be tabulated in various ways (Table I). There is some question as to whether these particular numbers could be reproduced because the one-two dozen cockroaches used were not truly randomized and we noted that one individual would yield only single and paired spores whereas the next one might give a high percentage of multiple groups. Perhaps such individual differences are due to the age of the plant or (more likely, we think) due to the length of the time interval since protruding spores were last brushed off. However, the important points are independent of the above uncertainty. These are:

(1) The spore groups protruding from perithecia may be sufficiently adherent to remain together as a compact cluster in the stain droplet, or they may dissociate into several (up to 4) smaller groups, even to single spores.[4] In column

TABLE I

Tabulation of data from 128 protruding spore groups picked off the tips of perithecia (columns 1 plus 2), and from the 188 spore groups into which these separated in the stain droplets (columns 5 plus 6). Total number of spores 362

Number of spores per group	From 128 perithecia		Total spores per group		From 188 adhering groups		Total spores per group	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
1	14	11	14	4	75	40	75	21
2	62	48	124	34	82	44	164	45
3	10	8	30	8	10	5	30	8
4	32	25	128	35	16	9	64	18
5	5	4	25	7	3	1.6	15	4
6	1	0.8	6	1.7	1	0.5	6	1.7
7	1	0.8	7	2	0	0	0	0
8	2	1.6	16	4.4	1	0.5	8	2.2
12	1	0.8	12	3.3	0	0	0	0

1, the numbers found per stain droplet (= per perithecium) are listed; in column 5 the numbers found per association group are listed. The 128 perithecia actually gave 188 groups in the stain. Perhaps a similar dissociation could occur on transference to the surface of another host (see below).

(2) The ejection of single spores is common.

(3) Most of the spore groups consisted of 1–4 spores but higher numbers up to 12 were occasionally found.

(4) The values shown in columns 1–4 of the table do not represent any common form of distribution; they are probably the product of several ejections preceding dislocation of the group (see below). In contrast, the values in columns 5–6 (listings for 188 adhering groups) do represent a skew-form distribution

[4] Sets of two were usually pairs but commonly two singles; sets of three were either 3 or 2 + 1 or 1 + 1 + 1; sets of four were 50% tetrads but sometimes 2 + 2 or less often 2 + 1 + 1 or 1 + 1 + 1 + 1; sets of five consisted of 5 or 3 + 1 + 1 or 2 + 2 + 1 or 2 + 1 + 1 + 1; the set of six consisted of 4 + 2; the set of seven was 4 + 2 + 1; the sets of eight were one set of 8 and one of 3 + 2 + 2 + 1; and the set of twelve was 6 + 5 + 1.

curve commonly encountered in biology; they also give a closer approximation to what one can see on the surface of cockroaches from infected colonies.

Another important point not shown by the table is that the aperture in the perithecium is not of sufficient diameter to permit the simultaneous passage of groups of 8 or 12 spores, and is questionably adequate for the simultaneous passage of a tetrad. Probably the answer is to be deduced from the observation that perithecia under coverglass pressure in a drop of water may be seen to eject spores like bullets coming out of a machine gun. They are ejected too rapidly for accurate determination of how many emerge at once but under these conditions they seem to come out singly. The important point, however, is that they are all ejected for about the same distance and hence align to form a cluster which to visual inspection may appear to be as tight a pack of adhering spores as if they had been ejected

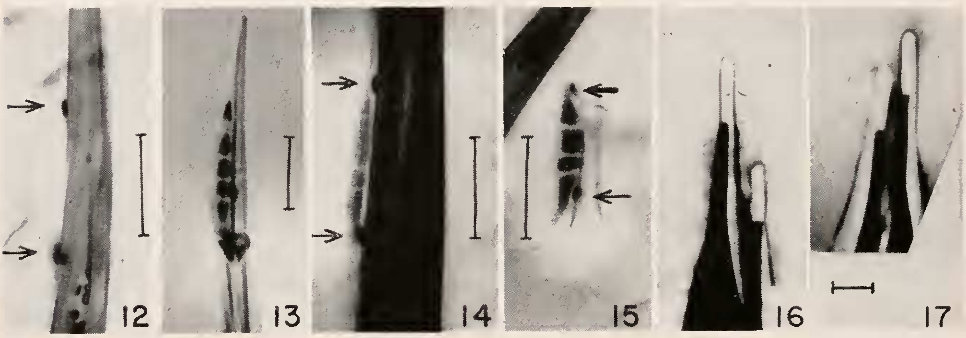

FIGURE 12. Part of a setal shaft from antenna of *Blatta orientalis* showing the two spore holdfasts (arrows) of *H. stylopygae* after dislodgment of the spore during preparation of slide. Cotton-blue stain. Bar 10 μ.

FIGURE 13. Spore of *H. ectobiae* in 4-cell stage on antennal seta of *Blattella germanica*. Note that there is a pair of basal holdfasts; presumably one spore of a pair became dislodged. Cotton-blue stain. Bar 10 μ.

FIGURE 14. Spore of *H. stylopygae* in 4-cell stage on an antennal seta of *Blatta orientalis*. Shows basal and apical holdfasts (arrows) but the sheath is almost invisible. Bar 10 μ.

FIGURE 15. Germinating spore of *H. stylopygae* on surface of antenna of *Blatta orientalis*. Note characteristic method of growing over the basal holdfast. Cotton-blue stain. Bar 10 μ.

FIGURES 16 AND 17. Electron micrograph of ends of two spores of *H. stylopygae*. The line in the underlying formvar membrane shows the original position and shape of the end of the spores. Under electron bombardment the spores have shrunk back and developed blunt ends (opaque). Bar 1 μ.

as a group. We suspect that ejection from the perithecia of *H. stylopygae* is of single spores and that groups of pairs or more represent two or more ejections which adhere because of the sticky external coat (said to be "gelatinous").

Examination of amputated antennae or other areas of the body wall, or of shed skins (exuvia), stained and cleared with cotton-blue in lacto-phenol and examined as whole mounts show large numbers of single and paired spores both on setae (Figs. 13–14) and on the body wall proper (Figs. 3 and 15). Larger groups are uncommon but occasionally seen. These data suggest that the numerous single plants arise from the development of single spores, and that at least some of the large plant clusters commonly seen arise from the development of multiple spore clusters.

Incidentally, both male and female plants develop to maturity in isolation; presumably the crowding permits fertilization of such female plants by nearby males. Single and paired spores (Fig. 13) as well as single (Figs. 4 and 6) and paired (Fig. 5) plants were also found with *H. ectobiae*.

Spores are normally and probably always discharged in the 2-cell stage. All of the 362 spores of Table I were 2-celled. Suspended in distilled water (Fig. 11) they measure $2\text{–}3 \times 20\text{–}30\ \mu$. Likely these spores are somewhat enlarged by uptake of water. A dried spore examined in the electron microscope measured $1.4 \times 25\ \mu$ with the bluntly rounded tips (Figs. 16–17) measuring $0.3 \times 2.5\ \mu$. These spores are colorless when ejected and have a thin wall. Both nuclei and cytoplasm are acidophilic in staining and gram-positive (Fig. 7). In the living state the clear nuclei are about $2\ \mu$ in diameter and are surrounded by small granules a small fraction of a micron in diameter (Fig. 11). The birefringent walls of the spore show in electron micrographs microfibers a few hundreths of a micron in diameter—a common microfiber diameter for chitinous membranes (Richards, 1951).

On the host the spores quickly become 4-celled and then usually show a brown or black spot at each end (Figs. 12–15). There is little or no visible difference between the two ends of the spores in either the 2-cell or 4-cell stages. That the brown or black spots at each tip serve as holdfasts, not previously reported to be present in any species of Laboulbeniales, is shown by the fact that when a spore becomes dislodged during preparation of a lacto-phenol mount these blobs may remain stuck firmly to the body wall or seta (Fig. 12). In the latter part of the 4-cell stage the germinating spores do show a longitudinal differentiation (Fig. 15).

Quite possibly the spores always emerge from the perithecium in one orientation but it does not seem reasonable to assume that with a brushing or dusting dissemination they always become applied to setae or the general body wall in one particular orientation. On setae they are always parallel to the longitudinal axis of the seta, as would be dictated by the action of surface forces, but on the general body wall they are oriented in all directions parallel to the surface. Yet on setae the plants always develop with the base at the proximal end of the seta, and on the body wall the apices of the plant project distally in relation to the antenna. It seems necessary to assume that either the orientation of the developing plant is somehow imposed by its position on the insect's cuticle, or that only those plants appropriately oriented develop (those in reverse orientation not developing and so contributing to the number of ungerminated 4-cell spores always present). Incidentally, artificially germinated spores also show visible differentiation of the two ends shortly after the 4-cell stage, as will be treated in a subsequent paper.

Volume Increase During Growth of H. stylopygae

Assigning appropriate geometric figures to the various stages one can calculate approximate volumes and hence volume increase during growth. Cigar-shaped spores, $2 \times 25\ \mu$, have a volume of approximately 25 cubic microns. Mature female plants calculated as a combination of cones, cylinders and elliptical spheroids have a volume 90,000 to 400,000 cubic microns, depending primarily on the number and size of the perithecia. The most gigantic female plants with haustorial bulbs $40 \times 100\ \mu$ would have a volume of approximately 500,000 cubic

microns. The portion of the plant visible on the surface of the insect, *i.e.*, plant minus haustorium, is considerably smaller, averaging 75,000 to 350,000 cubic microns. (Details of the structure of haustoria are given in part 2 of this series.)

The volume of a female plant is, then, 3500 to 20,000 times that of the spore from which it grows. Male plants are much smaller. Rough calculations from illustrations and measurements given in Thaxter's monographs show that other species of Laboulbeniales increase by $300 \times$ to $> 100{,}000 \times$ the spore size (not including the volume of haustoria presumably present). Obviously a considerable amount of nourishment must be obtained from the host even if we assume that the average density of the spore is considerably greater than that of the mature plant.

Loss of the Infection During Molting

In most cases (Diptera, Coleoptera, Hymenoptera), Laboulbeniales have been described from adult insects which do not molt. But in cockroaches they are found both on adults and the similar-appearing cohabiting nymphs. In adults the infections seem to survive until the insect dies, and in some cases Thaxter has described what he considered to be age changes in certain species. In nymphs of both *Blatta* and *Blattella* the infection is completely lost at ecdysis (molting). The external portions of the plant are visible on the exuvia (shed skin) and appear normal until they dry up.

In a direct test, 18 nymphs of *B. orientalis* were caught in the act of molting. The exuviae were checked and the infections mapped. The individuals were isolated and checked at intervals for 22 days ($> 50\%$ longer than the usual life cycle of the fungus). In no case did any infection develop. Many more have been tested indirectly by our finding that the most convenient method for getting known-to-be-uninfected cockroaches is to segregate into a clean container specimens by chance caught molting in our cultures. To the best of our knowledge this phenomenon of the complete loss of infection by molting has not previously been reported.

The failure to regenerate an infection after the thalli have been lost at molting shows that the large haustorial bulbs (see part 2) which must be left behind in the cockroach's epidermis cannot regenerate the lost plants. We are inclined to correlate this with the absence of nuclei from the haustoria but obviously other factors could be involved.

We have never seen any indication of the development of resistance to infection. Infected individuals freed of their infection by molting seem to be just as readily infected as ones not previously exposed to the fungus. In fact, in heavily infested colonies, most of the individuals have been re-infected in each instar and yet the really heavy infections develop on adults. Also, the re-infection may occur immediately because we have obtained some infected individuals after segregating specimens of *B. orientalis* which had molted so recently that their cuticles were still white (*i.e.*, $<$ one hour after molting).

Notes on H. ectobiae Thaxter

H. ectobiae growing on *Blattella* (= *Ectobia*) *germanica* has not been studied as intensively. Some differences from the habits of *H. stylopygae* have been noted.

Infections with *H. ectobiae* seem always to start on setae (Figs. 4–6). Usually they grow to the setal base and make an additional haustorial penetration there (Figs. 4–5). When they spread across the antennal surface larger plants result (Fig. 5). Of the hundreds we have examined no one has been seen in such a position that it could not have started development on a seta. We have noticed that male plants are more likely to reach large size without attaining the setal base than female plants are (Fig. 6). Female plants seem always to attain the setal base prior to maturing perithecia (Figs. 4–5).

Unlike *H. stylopygae, H. ectobiae* develops on any part of the surface of its host though nowhere attaining the density of heavy infections on antennae of *Blatta* and *Blaberus*. There is no evident pattern to the infection and no obvious differences correlated with sex of the host. Systematic survey with tabulation of infected areas on 34 individuals of *B. germanica* showed mature plants on antennae, maxillary and labial palpi, frons, coxae, femora, tibiae and tarsi of all pairs of legs, wings, thorax, abdomen (dorsal, lateral and ventral), and cerci. Some individuals had plants growing simultaneously on most of the above regions. Nymphs of *Blattella* commonly develop heavier infections than nymphs of *Blatta* do.

The spores of *H. ectobiae* regularly have a brown or black spot at each end in both the 2-cell and the 4-cell stage (Fig. 13); at least the proximal one of these appears to serve as a holdfast.

Summary

1. *Herpomyces stylopygae* Speg. is shown to be distinct from *H. periplanetae* Th. by host specificity tests.

2. Spores of *H. stylopygae* are found all over the surface of oriental cockroaches but mature plants are mostly found on the antennae, seldom on palpi and only rarely elsewhere. They grow on setae or on hard or soft cuticle but only on a living cockroach. Infections are heavier on males and on adults and experiments show the infection is disseminated by contact.

3. The ascus contains 8 spores which it liberates within the perithecium through a terminal perforation, leaving the ascus as a fluid-filled ghost.

4. Spores are ejected from the perithecia in various numbers, not just in pairs (Table I). Mostly the groups protruding from the subterminal apertures of the perithecia (Fig. 2) consist of 1–4 spores but groups as large as 12 spores were found. The presence of single, paired and multiple spore groups protruding from perithecia and found on the surface of hosts is correlated with the presence of single, paired and multiple plants on infected cockroaches.

5. Antennae of infected cockroaches serve as efficient spore brushes and dusters.

6. Spores become firmly attached to the cockroach's cuticle by holdfasts developed at both ends.

7. Development from spore to mature perithecia takes nearly two weeks.

8. The volume of a female plant is 3500–20,000 times that of a spore. So much material cannot be obtained from a minute volume of cuticle. A tubular haustorium through the cockroach's cuticle was found to expand into a large bulb in the epidermal cell layer.

9. Infections on adults persist but infections on nymphs were found to be completely lost when the nymph molts. The fungus plants are found intact on the shed skin.

10. There appears to be no development of resistance since individuals freed of infection by molting can be readily reinfected.

11. Some notes are given on spore structure, and on differences shown by *H. ectobiae.*

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